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Homotypic and Heterotypic Serum Neutralizing Antibody Response to Rotavirus Proteins Following Natural Primary Infection and Reinfection in Children

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Worldwide trials of rotavirus vaccines are currently in progress, but the basis of cross-reactive immunity between rotavirus serotypes is yet to be elucidated. The involvement of the outer capsid proteins, VP7 and VP4, in the production of cross-reactive neutralizing antibody (N-Ab) is unclear, and may be important for the success of animal rotavirus-based candidate vaccines that lack a VP4 of human rotavirus origin. In this study, VP7- and VP4-specific N-Ab was assayed in sera from children experiencing primary (27 children) and/or secondary (14 children) rotavirus infections using human-animal reassortant strains. These reassortants contained genes encoding the major G- and P-types found in human infection, including G1, 2, 3, and 4; or P1A[8], 1B[4], and 2[6]. After primary infection, the N-Ab response to VP7 was generally serotype-specific, whereas the response to VP4 was heterotypic. After reinfection (with the same or different serotypes) there was a significant increase (P = 0.0313) in the number of VP7 serotypes seroconverted against with no broadening of cross-reactivity to VP4. Increases in homotypic N-Ab titer, following both primary and secondary infection, were greater against VP7 than VP4, with the seroconversion against VP7 being significantly greater upon reinfection than following primary infection (P = 0.0280). In summary, heterotypic N-Ab produced following primary infection appears to be primarily against VP4. However, upon reinfection, VP7 becomes increasingly immunodominant both in terms of cross-reactive N-Ab production and increases in N-Ab titer. ***J. Med. Virol. 57:204–211, 1999.***

KEY WORDS: immunity; neutralization; rotavirus; reassortant; VP7; VP4

INTRODUCTION

Group A rotaviruses (RV) are important causative agents of acute dehydrating diarrhea in young humans and animals, resulting in mortality and morbidity of great social and economic significance. Antigenically the rotavirus virion is quite complex, with numerous levels of serological characterization. The inner capsid protein VP6, although not a proven mediator of antibody-based neutralization in humans, is an important immunogen by which isolates can be classified into serological subgroups. The outer capsid proteins, VP4 and VP7, are both neutralization antigens, with numerous serotypes of each circulating in the community. Various conventional associations of the major VP7, VP4, and VP6 antigenic types have been described [Gouvea and Brantly, 1995] and are the dominant consideration in the strategy for an effective vaccine.

There are four major VP7 (G) and three major VP4 (P) serotypes found in human clinical isolates. Overall, there have been 10 G-types and 8 P-types identified among human strains, with 21 different G- and P-type combinations so far confirmed [Estes, 1996; Hoshino and Kapikian, 1996]. This is in contrast to other animal groups, such as bovine and porcine RV strains, in which only six and eight different G- and P-type combinations, respectively, have so far been identified [Estes, 1996; Hoshino and Kapikian, 1996]. In general, G-types cross host boundaries quite readily, while P-types tend to be more restricted in host range, reflecting the role of VP4 in cell tropism and host range specificity [Kantharidis et al., 1988; Ramig and Galle, 1990]. However, these P-type host restrictions observed in

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animal strains are not as evident in human strains, where P-types related to ovine, porcine, feline, canine, simian, and bovine strains have been identified in human clinical isolates [Estes, 1996; Hoshino and Kapikian, 1996]. The human population seems to be an effective melting pot for RV reassortment perhaps due to close interactions with many different domesticated animal groups. Thus, the production of cross-reactive neutralizing antibody (N-Ab) against both VP4 and VP7 may be important for a protective response to natural infection and necessary for efficacy of the animal rotavirus-based vaccines currently being examined in humans.

Protection against RV infection is likely to be dependent on the gut mucosal immune response [Coulson et al., 1992; Matson et al., 1993]. Secreted IgA can be measured in feces but coproantibody assays are very difficult to standardize [Challacombe, 1995; Ferguson et al., 1995]. An alternative is the analysis of neutralizing Ab in serum. This is not necessarily a measure of protection against RV infection, but may identify important immunogens and map changes in responses after primary infection and later reinfection.

Studies of the serum antibody response to the neutralizing antigens following primary RV infection in humans have examined the relative immunodominance of VP4 and VP7 [Brüssow et al., 1990; Offit et al., 1993; Richardson et al., 1993; Ward et al., 1993]. In three of these studies, Ab directed at VP4 was found more often and/or to higher titer than Ab against VP7. Studies of serotype-specific (homotypic) and cross-reactive (heterotypic) Ab responses have concentrated on assay of Ab to VP7 [Brüssow et al., 1988a, 1988b; Taniguchi et al., 1991; Ward et al., 1992; Arias et al., 1994; O'Ryan et al., 1994; Rojas et al., 1995]. Most studies have found broadening of the cross-reactive VP7 Ab response following reinfection. Very little information has been provided regarding VP4-specific cross-reactive Ab production. Serum from infants after vaccination with human-animal rotavirus reassortants, including the simian RRV-based quadrivalent [Perez-Schael et al., 1994] and the bovine WC3-based [Christy et al., 1993] vaccines, generally show greater N-Ab responses to the VP4 of animal parent strains than against the human RV (HRV) VP7 component of the vaccines. Many studies of serum rotavirus-specific antibody have used nonfunctional assays, including epitope-blocking enzyme immunoassays (EIA) incorporating N-MAbs or radioimmunoprecipitation assays. These methods detect binding rather than neutralization, allowing limited extrapolation of the data to represent biologically significant events.

During the course of longitudinal surveillance studies, we identified children with primary and secondary rotavirus infections of known G- and P-types. We have examined the VP7 and VP4 N-Ab responses to homotypic and heterotypic G and P serotypes in sequential serum samples from these children after primary infections (27 children) and reinfections (14 children). Neutralization assays were performed using human-animal reassortant rotavirus strains representative of each of the major G- and P-types found in human clinical isolates. Comparisons of N-Ab titers in acute (or preinfection) sera and convalescent sera give a clearer picture of homotypic and heterotypic VP4 and VP7 serum antibody responses after natural rotavirus infection than has previously been described in young children.

TABLE I. Rotavirus Standard and Reassortant Strains Used for N-Ab Assays

Virus strain	VP7 origin	G serotype	VP4 origin	P sero [geno]type	Subgroup
Wa	Wa	1	Wa	1A[8]	II
rWa-4	SA11	3	Wa	1A[8]	II
rWa-7	Wa	1	SA11	[2]	II
RV5	RV5	2	RV5	1B[4]	I
rRV5-4	SA11	3	RV5	1B[4]	I
rRV5-7	RV5	2	SA11	[2]	I
RV3	RV3	3	RV3	2[6]	II
rRV3-7	RV3	3	TFR41	9[7]	I
ST3	ST3	4	ST3	2[6]	II
rST3-4	SA11	3	ST3	2[6]	II
rST3-7	ST3	4	SA11	[2]	I
SA11	SA11	3	SA11	[2]	I
TFR41	TFR41	5	TFR41	9[7]	I

MATERIALS AND METHODS

Viruses

Rotavirus standard and reassortant strains used in this study are listed in Table I. All viruses were propagated in MA104 cell monolayers. Reassortant rotavirus strains were produced and antigenically characterized as described previously [Gorrell and Bishop, 1997]. All reassortants other than rRV3-7 were produced by reassortment of HRV strains with the simian strain SA11, and their neutralization proteins (VP4 and VP7) have been shown to be unaffected antigenically by reassortment. As SA11 and RV3 share the same G serotype, serotype-specific N-MAbs would not discriminate between the RV3 and SA11 VP7s for selection of the reassortant representing HRV VP7 serotype G3. Therefore, rRV3-7, produced by reassortment of HRV strain RV3 and porcine strain TFR41 (VP7 serotype G5), was obtained from Dr. I.H. Holmes (Department of Microbiology, University of Melbourne). Using a panel of VP7- and VP4-specific neutralizing MAbs with reference to RV3 and TFR 41, the antigenic reactions of the RV3 VP7 in the TFR 41 background were shown by neutralization assays to be unaltered by reassortment.

Reassortant Virus Nomenclature

Nomenclature used for all HRV × animal RV reassortant strains in this study was in the form of rHRV-4 or rHRV-7, where r denotes that the strain is a reassortant, HRV denotes the human rotavirus parent (Wa, RV5, RV3, or ST3), and the neutralization antigen of HRV origin (VP4 or VP7) is denoted as -4 or -7.

Patient Samples

Sequential serum samples were obtained from 30 children recruited at birth and screened for the absence

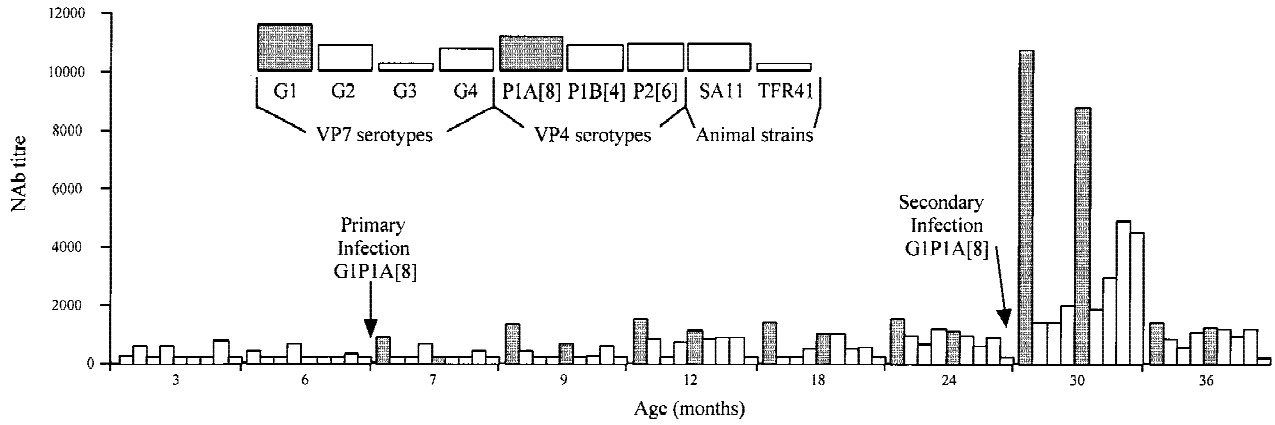

Fig. 1. Neutralizing antibody titers against reassortants representing each of the major human rotavirus VP7 (G) and VP4 (P) serotypes in sequential sera from a child experiencing primary and secondary rotavirus infections. The insert illustrates the order (left to right) of N-Ab titers against human × animal reassortants rotavirus strains, representing individual VP7 and VP4 antigens, and against the parent animal strains in each serum sample. Shaded bars represent homotypic reactions directed against VP7 and VP4 serotypes of the infecting strains.

of neonatal rotavirus infection (15 children) [Bishop et al., 1983] or recruited at 1–28 months of age (15 children) after admission to hospital with severe diarrhea due to rotavirus infection [Grimwood et al., 1988]. Twenty-nine of the children were kept under surveillance for 18–36 months and one child was followed for 2 months only. Rotavirus infections were diagnosed by EIA [Coulson et al., 1987] and/or RT-PCR assay [Gouvea et al., 1990] of weekly stool specimens; EIA estimation of antirotavirus IgA in weekly stool specimens; EIA antirotavirus IgG seroconversion in sera obtained at 3–6 months intervals. The G- and P-types of rotaviruses identified in stools were assigned by EIA [Grimwood et al., 1988] and/or RT-PCR [Gouvea et al., 1990; Gentsch et al, 1992].

Overall, 263 serum samples (>6 specimens from most children) were assayed for neutralizing antibody to each of the standard and reassortant rotavirus strains listed in Table I.

Neutralizing Ab Assays

Serum N-Ab produced in response to natural rotavirus infection was measured by a neutralization assay as described previously [Gorrell and Bishop, 1997]. Briefly, 10-fold serial dilutions of serum were incubated with each virus strain for 1 hr at 37°C. The serum/virus mix (or virus alone) was transferred onto MA104 cell monolayers and incubated overnight. Viral antigen was detected after lysis of the monolayers by freeze-thawing followed by assay in a rotavirus-specific EIA. N-Ab titer was defined as the reciprocal serum dilution resulting in a 50% reduction in virus-specific absorbance.

Seroconversion Indexes

For the purpose of simplification, N-Ab titers against each VP7 (G) and VP4 (P) type were converted to a seroconversion index representing the fold increase in N-Ab titer in paired sera (provided that seroconversion to the corresponding HRV parent strain of the reassortant was also detected). Seroconversion indexes were mathematically determined as the highest postinfection N-Ab titer against each virus (obtained from either convalescent or late convalescent sera) divided by the N-Ab titer in preinfection or acute sera for the same virus. Calculation of seroconversion indexes allowed interpatient and interantigen comparisons and analysis of secondary infection independently of the primary immune response.

Statistical Analysis

Statistical tests used to analyze results include Wilcoxin signed-rank test to test the equality of matched pairs of observations; two-sample Wilcoxin rank-sum (Mann-Whitney) test to test the hypothesis that two independent samples are from populations with the same distributions; sign test to test the equality of matched pairs of observations; and a nonparametric test to test for trends across ordered groups [Altman, 1991].

Ethical Approval

Written informed consent for surveillance was obtained from all parents. Ethical approval for the study was granted by the Ethics in Human Research Committee of the Royal Children's Hospital.

RESULTS

Neutralizing antibody titers against all viruses listed in Table I were determined for each serum sample collected longitudinally from children naturally infected with rotavirus. Of the 30 children studied, sera was available following primary infection only, both primary and secondary infections or secondary infection only, for 16, 11, and 3 children, respectively. The N-Ab titer results from sequential sera of one representative patient infected on two occasions with a G1P1A[8] strain is shown (Fig. 1). In order to simplify the figure, results from sera obtained at 15, 21, 42, and 48 months of age were excluded. In each case, N-Ab titers from

TABLE II. Neutralizing Antibody Seroconversions to Reassortant Rotaviruses Following Natural Primary Rotavirus Infection

Infecting strain G-, P-type	Patient number	Age (months)	Serconversion index[a]						
			VP7 types				VP4 types		
			G1	G2	G3	G4	P1A[8]	P1B[4]	P2[6]
G1, P1A[8]	1	1	**5**	.	.	.	**4**	.	.
	2	2	.	.	.	.	.	.	.
	3	3	.	.	.	.	.	.	.
	4	7	.	4	.	.	**6**	.	5
	5	8	**8**	.	.	.	**6**	5	4
	6	9	**6**	.	.	.	**6**	5	5
	7	10	**10**	6	.	.	**23**	.	6
	8	11	.	.	.	.	**9**	.	.
	9	11	**37**	.	.	5	**22**	7	4
	10	15	**7**	.	.	4	**5**	.	.
	11	17	**6**	.	.	.	**5**	6	7
	12	20	.	.	.	.	**6**	.	5
	13	21	**7**	.	.	5	**25**	8	.
	14	22	**5**	.	.	.	**5**	.	.
	15	23	.	.	.	.	**6**	.	5
	16	25	**10**	4	.	5	**6**	.	5
	17	28	**21**	.	.	7	**8**	17	.
G2, P1B[4]	18	2	.	**5**	.	.	.	.	.
	19	2	.	**5**	.	.	.	**5**	.
	20	8	.	**9**	.	.	.	**5**	.
	21	12	.	.	.	.	.	**5**	.
G3, P1A[8]	22	24	.	.	.	.	**8**	.	5
G4, P1A[8]	23	9	7	.	.	**7**	**5**	.	5
	24	10	5	.	.	**42**	**11**	6	28
	25	17	.	.	.	**5**	.	.	4
	26	21	.	.	.	**8**	**6**	5	5
	27	21	.	.	.	**7**	**27**	4	7

[a]Seroconversion index = highest postinfection N-Ab titer ÷ acute or preinfection N-Ab titer. Boldface denotes homotypic serotype response. Period (.) indicates <fourfold increase in N-Ab titer (no seroconversion).

these sera remained similar to those from the preceding sera. Primary infection resulted in initial increases in N-Ab titer predominantly homotypic to the infecting strain, although increases in N-Ab to most G- and P-types were present during the following 17 months. Reinfection resulted in a further rise in N-Ab titer to all G- and P-types examined, including an animal virus (TFR41) bearing nonhuman G- and P-type determinants.

N-Ab Response Following Primary Rotavirus Infection

Table II lists the results of N-Ab titrations against the major HRV VP7 and VP4 serotypes for sequential sera from 27 children experiencing primary rotavirus infections. All results are expressed as seroconversion indexes. Of the children with G1P1A[8] infections, 11/17 (65%) showed homotypic seroconversion to VP7 and 15/17 (88%) showed homotypic seroconversion to VP4. Only two children (infants aged 2 and 3 months) showed no detectable seroconversion to either VP7 or VP4. Heterotypic VP7 (to G2 and/or G4) and VP4 (to P1B[4] and/or P2[6]) seroconversions were detected in 7/17 (41%) and 11/17 (65%) children, respectively.

All five children with G4P1A[8] infections seroconverted to the homotypic G4, with two also seroconverting to G1 VP7. All children concurrently showed heterotypic VP4 seroconversions. The four children with G2P1B[4] infections all seroconverted to the homotypic serotypes only. The child with a G3P1A[8] infection seroconverted only to VP4. Seroconversion to one of the animal strains, SA11 or TFR41, was detected in eight (30%) and two (7%) of the primary infection children, respectively. No children seroconverted to both animal strains.

With respect to VP4 vs. VP7 immunodominance, more children (48%) seroconverted to a greater number of P-types than G-types, while 11% seroconverted to more G-types than P-types. However, homotypic seroconversion indexes were more often greatest against the VP7 (48%) than the VP4 serotype (33%).

N-Ab Response Following Reinfection With Rotavirus

Sera were available from 14 children in whom the G- and P-type of the strain responsible for reinfection were identified (Table III). Of the 12 children with G1P1A[8] reinfection, all seroconverted to the homotypic G1 VP7, with 10 (83%) also seroconverting to heterotypic VP7 serotypes. Ten (83%) of the 12 children seroconverted to VP4, with 8 (67%) showing both homotypic and heterotypic N-Ab seroconversions. The two G4P1A[8]-infected children seroconverted both homo- and heterotypically to VP4, but only one child showed heterotypic VP7 seroconversion as well as the homotypic G4 seroconversion. Twelve (86%) and 9

TABLE III. Neutralizing Antibody Seroconversions to Reassortant Rotaviruses Following Natural Secondary Rotavirus Infection

Reinfection strain G-, P-type	Patient number	Age (months)	Serconversion index[a] VP7 types G1	G2	G3	G4	VP4 types P1A[8]	P1B[4]	P2[6]
G1, P1A[8]	1	16	**11**	5	.	.	.	.	.
	2	18	**20**	.	.	53	**10**	.	13
	3	18	**100**	15	.	7	**11**	5	.
	4	30	**7**	.	.	.	**8**	.	5
	7	45	**8**	5	.	5	**7**	.	5
	11	21	**10**	.	7	.	6	.	.
	15	41	**5**	.	.	.	.	8	.
	19	14	**8**	11	6	21	**60**	17	10
	25	43	**43**	.	40	26	**63**	46	37
	26	25	**5**	4	.	.	.	.	.
	28	10	**9**	.	9	.	**9**	9	10
	29	25	**52**	36	9	22	**10**	41	6
G4, P1A[8]	18	13	.	.	.	**6**	**6**	8	.
	30	20	7	6	44	**9**	**16**	12	7

[a]Seroconversion index = highest postinfection N-Ab titer ÷ acute or preinfection N-Ab titer. Boldface type denotes homotypic serotype response. Period (.) indicates <fourfold increase in N-Ab titer (no seroconversion).

TABLE IV. Neutralizing Antibody Seroconversions to Reassortant Rotaviruses Following Paired Primary and Secondary Rotavirus Infections

Patient number	VP7 serotype N-Ab seroconversion[a] Primary infection G1	G2	G3	G4	Reinfection G1	G2	G3	G4	VP4 serotype N-Ab seroconversion Primary Infection P1A[8]	P1B[4]	P2[6]	Reinfection P1A[8]	P1B[4]	P2[6]
1	+	.	.	.	+	+	.	.	+	.	.	.	.	.
2	.	.	.	.	+	.	.	+	.	.	.	+	.	+
3	.	.	.	.	+	+	.	+	.	.	.	+	+	.
4	.	+	.	.	+	.	.	.	+	.	+	+	.	+
7	+	+	.	.	+	+	.	+	+	.	+	+	.	+
11	+	.	.	.	+	.	+	.	+	+	+	+	.	.
15	.	.	.	.	+	.	.	.	+	.	+	.	+	.
25	.	.	.	+	+	.	+	+	.	.	+	+	+	+
26	.	.	.	+	+	+	.	.	+	+	+	.	.	.
19	.	+	.	.	+	+	+	+	.	+	.	+	+	+
18	.	+	.	.	.	.	.	+	.	.	.	+	+	.

[a]Cross (+) denotes seroconversion (≥fourfold increase in N-Ab titer). Boxed areas indicate homotypic VP7 and VP4 serotypes of infecting strains (primary and secondary).

(64%) of the 14 reinfected children also seroconverted to the animal strains SA11 and TFR41, respectively. All children that seroconverted to TFR41 also seroconverted to SA11.

With respect to VP4 vs. VP7 immunodominance following reinfection, only 21% of children seroconverted to a greater number of P-types than G-types, while 58% seroconverted to more G-types than P-types. Seroconversion indexes against homotypic G-types remained more often greater than those against homotypic P-types (57%) than the converse (29%), as seen following primary infection.

Comparison of N-Ab Response Following Primary and Secondary Rotavirus Infections

For 11 of the children studied, serum from both primary and secondary rotavirus infections were available (Table IV). Of these children, seven were infected sequentially with strains of the same VP7 and VP4 serotypes (patients 1, 2, 3, 4, 7, 11, 15), two were infected with strains of different VP7 serotype but same VP4 serotype (patients 25, 26), and two were infected with strains different in both VP7 and VP4 serotype (patients 18, 19). Disease was less severe in all children at reinfection than during their primary infection, and asymptomatic reinfections occurred regardless of whether reinfection was with a strain of identical or different serotype to the primary infection.

Nine (82%) of the 11 children seroconverted to an increased number of G-types following reinfection compared with primary infection. With respect to VP4, 5/11 (45%) children seroconverted to more P-types following secondary infection than their primary infection.

Figure 2 shows the frequency of seroconversion by all children examined in this study to nil, one, or multiple VP7 and VP4 serotypes following primary and/or secondary infection regardless of infection serotype. The 11 children from whom serum following paired primary and secondary infections were available showed a similar distribution of G- and P-type seroconversions (data

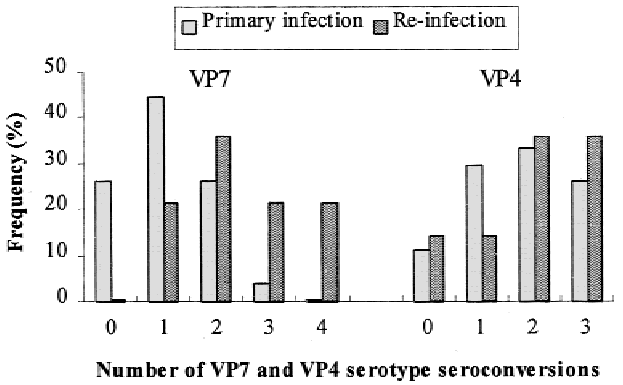

Fig. 2. Frequency of seroconversion to zero, one, and multiple VP7 and VP4 serotypes following primary and secondary natural rotavirus infection in all children studied.

not shown). Statistical significance of the observed shift in distribution of G-type seroconversions was achieved for G1P1A[8] secondary vs. G1P1A[8] primary infections only for both paired ($P = 0.031$, sign test) and unpaired ($P = 0.0035$, nonparametric test for trend across ordered groups) infection data. Other serotype groups were too small for statistical analysis. The paired G1P1A[8] infection results also showed a significant increase in seroconversion index following reinfection against homotypic VP7 type G1 ($P = 0.028$) and the animal strains SA11 ($P = 0.022$) and TFR41 ($P = 0.022$) using the Wilcoxin signed-rank test. There was no significant increase in seroconversion index against any other G-type or any VP4 serotypes. The unpaired data also showed a significant increase in G1 ($P = 0.013$), SA11 ($P = 0.0018$), and TFR41 ($P = 0.0002$) seroconversion indexes using the two-sample Wilcoxin rank-sum (Mann-Whitney) test.

DISCUSSION

Serum N-Ab responses in young children experiencing primary and secondary rotavirus infections were analyzed using human–animal rotavirus reassortant viruses representing each of the major HRV VP4 and VP7 serotypes. Extensive preliminary characterization of these reassortants had established that the antigenicity of the HRV VP4 and VP7 was unaltered compared with the complementary natural human strains [Gorrell and Bishop, 1997].

There were qualitative and quantitative differences between the N-Ab responses in sequential sera obtained following primary and secondary infections in young children. After primary infection, VP4 responses, both homotypic (85%) and heterotypic (63%), were more common than VP7 responses (70% and 33%, respectively). In most children, seroconversion indexes were higher for homotypic responses than for heterotypic responses. The failure of all four children with primary G2P1B[4] infections to develop heterotypic responses to either protein is in contrast to findings of other studies [Arias et al., 1994]. After reinfection all children seroconverted to VP7, with the VP7 N-Ab produced at reinfection being significantly more heterotypic ($P = 0.031$) than that after primary infection. This broadening cross-reactivity of VP7-specific Ab following reinfection has also been observed using other assays, including immunoprecipitation (Dr. S. Richardson, personal communication) and epitope blocking [Taniguchi et al., 1991]. Seroconversion indexes to the homotypic G serotype was also significantly greater following reinfection ($P = 0.028$). There was no significant difference in the VP4 N-Ab response following reinfection compared with primary infection with respect to the number of children seroconverting, seroconversion indexes, and cross-reactivity of N-Ab produced.

The markedly increased VP7 response observed following human reinfection, relative to primary infection and the VP4 response, may be associated with limited replication of the virus during reinfection compared with that occurring at primary infection [Mendis et al., 1993; Velazquez et al., 1996]. Intracellular neutralization of VP6 [Burns et al., 1996] and other mechanisms causing a reduction in replication and assembly of mature virions, together with rapid clearance of virus following reinfection [Franco et al., 1997], could influence epitope processing and presentation. Reduction in exposure to VP4 and presentation of previously hidden VP7 epitopes could occur. In addition, age-related differences in immune function, for example in ratio of IgA1 to IgA2 secretion in infancy vs. childhood [Friedman et al., 1996], could also influence the response to reinfection. Therefore, the biological mechanisms underlying the expanded VP7 response observed following reinfection may be related to viral or immune system influences, or components of both.

In addition to broadening responses to human viruses after reinfection, the study showed a significant increase in cross-reactive seroconversions with animal strains SA11 and TFR41($P = 0.022$ for each strain). The cross-reactivity detected with SA11 could be explained in one child by G3 neonatal infection (patient 28). All other children had no known exposure to G3 viruses. The TFR41 cross-reactivity detected upon reinfection was unexpected because, although G5P1A[8] strains have recently been reported in Brazilian children [Timenetsky et al., 1997], there is no evidence that the children in this study had any G5 rotavirus exposure. Bovine studies have shown that reinfection results in broadening of the immune response to include responses to human rotavirus serotypes are unlikely to have caused infection in the bovine population [Brüssow et al., 1988a, 1988b; Snodgrass et al, 1991]. These results suggest that shared cross-reactive epitopes are common but may require repeated infection for immune recognition. Further studies are required to identify cross-reactive epitopes on VP7, particularly aimed at virus G-types rarely encountered in natural human infection.

This study allowed limited comparison of the immune response to VP7 serotypes G1 and G4 in relation to the associated P1A[8] VP4. Infection with G4P1A[8] produced a heterotypic response to VP4 following pri-

mary infection more frequently than G1 infections, particularly with respect to P2[6] VP4. It is possible that G4-associated VP4 may be more likely to elicit P2[6] cross-reactive N-Ab than G1-associated VP4. Variation of alleles has previously been recorded within the P1A[8] serotype and has been associated with variable elicitation of cross-reactive N-Ab [Padilla-Noriega et al., 1993]. Influence by the infecting strain on the extent of heterotypic immunity has been suggested previously [Xu et al., 1993].

Recruitment and surveillance of naturally infected children differs in many ways from controlled animal experiments and vaccine trials where rotavirus strains used can be standardized. Strains causing natural human infections, even when of identical G and P serotypes, may vary in amino acid sequence identity [Wen et al., 1997]. A number of other issues must be considered when analyzing results of this nature. Recruits varied in age at onset of infection and reinfection. In particular, the onset date of reinfection, particularly if asymptomatic, was usually impossible to establish. As a result, serum samples may not have been obtained at optimal time postinfection for estimation of antibody levels. Unlike controlled studies of vaccine recipients, the initial infectious dose and therefore subsequent viral load were also unknown variables. Despite these drawbacks, the benefits of studying naturally infected children include the knowledge that the immune response being examined is against naturally occurring community strains replicating in and being shed by the host, and that these responses may be implicated in later protection against severe disease, i.e., the response to be emulated by vaccination.

The results of this study suggest that both VP4 and VP7 are important stimulators of circulating N-Ab, with VP4 providing a broader N-Ab response than VP7 following primary infection and VP7 becoming increasingly immunodominant with further rotavirus exposure. These results complement other studies, suggesting that for a rotavirus vaccine to be efficacious, it should be multidose in order to elicit cross-reactive VP7 N-Ab. However, serum Ab results are only predictive of the protective mucosal response to rotavirus infection in humans. Further analysis of coproantibody response and the mucosal cellular and cytokine response may be required for detailed elucidation of the protective immune response to natural rotavirus infection.

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REFERENCES

Altman DG, editor. 1991. Practical Statistics for Medical Research. London: Chapman and Hall.

Arias CF, López S, Mascavenhas JD, Romero P, Cano P, Gabbay YB, de Freitas RB, Linhares AC. 1994. Neutralizing antibody immune response in children with primary and secondary rotavirus infections. Clin Diagn Lab Immunol 1:89–94.

Bishop RF, Barnes GL, Cipriano E, Lund JS. 1983. Clinical immunity after neonatal rotavirus infection. N Engl J Med 309:72–76.

Brüssow H, Walther I, Fryder V, Sidoti J, Bruttin A. 1988. Cross-neutralizing antibodies induced by single serotype vaccination of cows with rotavirus. J Gen Virol 69:1647–1658.

Brüssow H, Werchau H, Lerner L, Mietens C, Liedtke W, Sidoti J, Sotek J. 1988. Seroconversion patterns to four human rotavirus serotypes in hospitalized infants with acute rotavirus gastroenteritis. J Inf Dis 158:588–595.

Brüssow HP, Offit A, Gerna G, Bruttin A, Sidoti J. 1990. Polypeptide specificity of antiviral serum antibodies in children naturally infected with human rotavirus. J Virol 4:4130–4136.

Burns JW, Siadat-Pajouh M, Krishnaney AA, Greenberg HB. 1996. Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. Science 272:104–107.

Challacombe SJ. 1995. Assessing mucosal humoral immunity. Clin Experiment Immunol 100:181–182.

Christy C, Offit P, Clark F, Treanor J. 1993. Evaluation of a bovine-human rotavirus reassortant vaccine in infants. J Inf Dis 168: 1598–1599.

Coulson BS, Unicomb LE, Pitson GA, Bishop RF. 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotavirus. J Inf Dis 25:509–515.

Coulson BS, Grimwood K, Hudson IL, Barnes GL, Bishop RF. 1992. Role of coproantibody in clinical protection of children during reinfection with rotavirus. J Clin Microbiol 30:1678–1684.

Estes MK. 1996. Rotaviruses and their replication. In Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, Roizman B, Strauss SE, editors. Fields virology, 3rd ed., vol. 2. Philadelphia: Lippincott-Raven, p 1625–1655.

Ferguson AK, Humphreys A, Croft NM. 1995. Technical report: Results of immunological tests on faecal extracts are likely to be extremely misleading. Clin Experiment Immunol 99:70–75.

Franco MA, Tin C, Greenberg HB. 1997. $CD8^+$ T cells can mediate almost complete short-term and partial long-term immunity to rotavirus in mice. J Virol 71:4165–4170.

Friedman MG, Entin N, Zedaka R, Dagan R. 1996. Subclasses of IgA antibodies in serum and saliva samples of newborns and infants immunized against rotavirus. Clin Experiment Immunol 103:206–211.

Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK. 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 30:1365–1373.

Gorrell RJ, Bishop RF. 1997. Production of reassortant viruses containing human rotavirus VP4 and SA11 VP7 to measure neutralizing antibody following natural infection. Clin Diagn Lab Immunol 4:509–514.

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang Z. 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 28:276–282.

Gouvea V, Brantly M. 1995. Is rotavirus a population of reassortants? Trends Microbiol 3:159–162.

Grimwood K, Lund JCS, Coulson BS, Hudson IL, Bishop RF, Barnes GL. 1988. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. J Clin Microbiol 26:732–738.

Hoshino Y, Kapikian AZ. 1996. Classification of rotavirus VP4 and VP7 serotypes. Arch Virol 12(suppl):99–111.

Kantharidis PM, Dyall-Smith L, Tregear GW, Holmes IH. 1988. Nucleotide sequence of UK bovine rotavirus segment 4: Possible host restriction of VP3 genes. Virology 166:308–315.

Matson DO, O'Ryan ML, Herrera I, Pickering LK, Estes MK. 1993. Fecal antibody response to symptomatic and asymptomatic rotavirus infections. J Inf Dis 167:577–583.

Mendis L, Senanayake S. 1993. Serum and coproantibody responses to rotavirus infection in children during the first two years of life. J Diarrheal Dis Res 11:75–81.

O'Ryan ML, Matson DO, Estes MK, Pickering LK. 1994. Acquisition of serum isotype-specific and G type-specific antirotavirus antibodies among children in day care centers. Pediatric Inf Dis J 13:890–895.

Offit PA, Hoffenberg EJ, Santos N, Gouvea V. 1993. Rotavirus-specific humoral and cellular immune response after primary, symptomatic infection. J Inf Dis 167:1436–1340.

Padilla-Noriega L, Werner-Eckert R, Mackow ER, Gorziglia M, Larralde G, Taniguchi K, Greenberg HB. 1993. Serologic analysis of human rotavirus serotypes P1A and P2 by using monoclonal antibodies. J Clin Microbiol 31:622–628.

Perez-Scael I, Blanco M, Garcia D, White L, Alfonzo E, Crespo I, Cunto W, Pittman AL, Kapikian AZ, Flores J. 1994. Evaluation of the antigenicity and reactogenicity of varying formulations of the rhesus rotavirus-based quadrivalent and the M37 rotavirus vaccine candidates. J Med Virol 2:330–337.

Ramig RF, Galle KL. 1990. Rotavirus genome segment 4 determines viral replication phenotype in cultured liver cells (HepG2). J Virol 64:1044–1049.

Richardson SC, Grimwood K, Bishop RF. 1993. Analysis of homotypic and heterotypic serum immune responses to rotavirus proteins following primary rotavirus infection by using the radioimmunoprecipitation technique. J Clin Microbiol 31:377–385.

Rojas AM, Boher Y, Guntinas MJ, Perez-Schael I. 1995. Homotypic immune response to primary infection with rotavirus serotype G1. J Med Virol 47:404–409.

Snodgrass DR, Fitzgerald TA, Campbell I, Browning GF, Scott FMM, Hoshino Y, Davies RC. 1991. Homotypic and heterotypic serological response to rotavirus neutralization epitopes in immunologically naive and experienced animals. J Clin Microbiol 29:2668–2672.

Taniguchi K, Urasawa T, Kobayashi N, Ahmed MU, Adachi N, Chiba S, Urasawa S. 1991. Antibody response to serotype-specific and cross-reactive neutralization epitopes on VP4 and VP7 after rotavirus infection or vaccination. J Clin Microbiol 29:483–487.

Timenetsky M do C, Gouvea V, Santos N, Carmona RC, Hoshino Y. 1997. A novel human rotavirus serotype with dual G5-G11 specificity. J Gen Virol 78:1373–1378.

Velazquez FR, Matson DO, Calva JJ, Guerrero ML, Morrow AL, Carter-Campbell S, Glass RI, Estes MK, Pickering LK, Ruiz-Palacios GM. 1996. Rotavirus infection in infants as protection against subsequent infections. N Engl J Med 335:1022–1028.

Ward RL, Clemens JD, Knowlton DR, Roa MR, van Loon FPL, Huda N, Ahmed F, Schiff GM, Sack DA. 1992. Evidence that protection against rotavirus diarrhea after natural infection is not dependent on serotype-specific neutralizing antibody. J Inf Dis 166:1251–1257.

Ward RL, McNeal MM, Sander DS, Greenberg HB, Bernstein DI. 1993. Immunodominance of the VP4 neutralization protein of rotavirus in protective natural infections of young children. J Virol 67:464–468.

Wen L, Nakayama M, Yamanishi Y, Nishio O, Fang Z-Y, Nakagomi O, Araki K, Nishimura S, Hasegawa A, Müller WEG, Ushijima H. 1997. Genetic variation in the VP7 gene of human rotavirus serotype 3 (G3 type) isolated in China and Japan. Arch Virol 142: 1481–1489.

Xu Z, Hardy ME, Williams JD, Woode GN, Ramig RF. 1993. Immunodominant neutralizing antigens depend on the virus strain during primary immune response in calves to bovine rotaviruses. Veterinary Microbiol 35:33–43.